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- (71) Applicant (*for all designated States except US*): **THER-APTOSIS S.A.** [FR/FR]; Pasteur Biotop, 25, rue du Dr. Roux, F-75015 Paris (FR).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **JACOTOT**, Etienne [FR/FR]; 171, rue Lecourbe, F-75015 Paris (FR). **LECOEUR**, Hervé [FR/FR]; 113, rue Didot, F-75014 Paris (FR). **REBOUILLAT**, Dominique [FR/FR]; 36, rue du Hameau, F-75015 Paris (FR).
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(54) Title: METHOD FOR SCREENING MODULATORS OF MITOCHONDRIAL FUNCTIONING

(57) Abstract: The invention relates to a method for screening modulators of mitochondrial function comprising adding a compound to be tested in a purified, isolated mitochondria preparation and simultaneously using fluorimetric analysis of mitochondrial morphology, and especially real-time fluorimetric analysis, combining analysis of morphometric parameters (SSC/FSC parameters) with analysis of membrane integrity by dye fluorescence. Application to the obtention of peptides which induce mitochondrial membrane permeabilization.

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## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 03/12056

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 G01N33/50 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, INSPEC, BIOSIS, MEDLINE, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PFEIFFER DOUGLAS R ET AL: "The peptide mastoparan is a potent facilitator of the mitochondrial permeability transition" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 9, 1995, pages 4923-4932, XP002279212 ISSN: 0021-9258 abstract table I  ----- -/--	1-9

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Tuynman, A

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 03/12056

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>MINAMIKAWA TETSUHIRO ET AL:            "Mitochondrial permeability transition and swelling can occur reversibly without inducing cell death in intact human cells"            EXPERIMENTAL CELL RESEARCH,            vol. 246, no. 1,            10 January 1999 (1999-01-10), pages 26-37,            XP002279213            ISSN: 0014-4827            the whole document</p>	1-9
X	<p>-----</p> <p>MINAMIKAWA T ET AL:            "Chloromethyl-X-rosamine (MitoTracker Red) photosensitises mitochondria and induces apoptosis in intact human cells."            JOURNAL OF CELL SCIENCE. ENGLAND JUL 1999,            vol. 112 ( Pt 14), July 1999 (1999-07),            pages 2419-2430, XP002279214            ISSN: 0021-9533            the whole document</p>	1-9
A	<p>-----</p> <p>WALDMEIER PETER C ET AL: "Inhibition of the mitochondrial permeability transition by the nonimmunosuppressive cyclosporin derivative NIM811"            MOLECULAR PHARMACOLOGY,            vol. 62, no. 1, July 2002 (2002-07), pages 22-29, XP002279215            ISSN: 0026-895X            abstract</p>	1-9
P,X	<p>-----</p> <p>MATTIASSEN GUSTAV ET AL: "Flow cytometric analysis of mitochondria from CA1 and CA3 regions of rat hippocampus reveals differences in permeability transition pore activation."            JOURNAL OF NEUROCHEMISTRY,            vol. 87, no. 2, October 2003 (2003-10),            pages 532-544, XP002279216            ISSN: 0022-3042            the whole document</p> <p>-----</p>	1-9

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP 03/12056

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9 fully

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-9 (fully)

1) methods for screening modulators of mitochondrial function (claims 1-9 fully).  
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2. claims: 10-12 (partially)

Synthetic peptides that induce cell death of various cell types (SEQ ID No 2-4,8,9,12-18,36-41).  
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3. claims: 10-12 (partially)

Synthetic peptides that induce cell death of adenocarcinoma cell lines (SEQ ID NO 10,11,24-35,42-53)  
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4. claims: 10-12 (partially)

Synthetic peptides that induce cell death of HUVECs (SEQ ID NO 5,20-23,54-57).  
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5. claims: 10,11 (partially)

Synthetic peptide according to SEQ ID No 1 as modulator of mitochondrial function.  
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6. claims: 10,11 (partially)

Synthetic peptide according to SEQ ID No 6 as modulator of mitochondrial function.  
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7. claims: 10,11 (partially)

Synthetic peptide according to SEQ ID No 7 as modulator of mitochondrial function.  
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8. claims: 10,12 (partially)

Synthetic peptide according to SEQ ID No 19 as modulator of mitochondrial function  
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